ENSCnews

The European native seed conservation newsletter

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editorial

A key strength of seed banking is that the technology is essentially simple. Seeds from plants that are threatened in the wild are collected and kept alive in a seed bank by means of drying followed by cooling. Access to dwindling and important wild plant diversity is assured through seed banks. However, in common with most other organisms, seeds are complex entities. Consequently, their response to the dry, cold conditions in a seed bank varies between species. Some species ('recalcitrant' and 'intermediate' types) are killed by drying. Fortunately, in Europe we have relatively few such species; nearly all species have 'orthodox' desiccation-tolerant seeds. In the case of most of these, storage lives of many decades, and probably very much longer, are predicted under seed bank conditions. As we learn more about the storage behaviour of seeds, however, it is apparent that acceptable longevity for seeds of a few 'orthodox' species is only achieved using very much colder conditions than the deep-freeze temperatures used by most banks. Such cryo-preservation is achieved using liquid nitrogen. One European bank that is using this technology is the ENSCONET partner at Warsaw Botanic Garden. Their approach is the focus of the first article.

The success of seed banking relates directly to the quality of staff involved. The protocols that underpin seed banking are simple and yet technicians need to be able to interpret them when faced with the dramatic range of plant diversity encountered. Scientific researchers need to test continually the effectiveness and efficiency of these protocols and ensure that their findings are translated into new techniques and equipment. Quite apart from seed science, work is needed on understanding related subjects such as ecology, genetics and horticulture. Managers of seed banks not only need to integrate all of these activities, but also to continually update their knowledge of the background in which the bank operates. This background includes changing collection use and bio-politics. Consequently, seed banks are much more than bottles of seeds. Article five gives the flavour of one week in the life of the UK's ENSCONET partner.

Europe is fortunate in having a great deal of seed bank expertise that should make a real impact in addressing the losses of the continent's native plant diversity and also has a leading role to play across the globe. However, this very expertise needs to be conserved. All too often, these long-term facilities limp from year to year on short-term and modest funding. Against a deteriorating financial situation in the private and public sectors, the financial security of Europe's seed banks looks uncertain. Without support, another banking crisis with even longer-term effects may be in the making.

Front Cover

The front cover shows germinating spores of the tropical fern *Cyathea australis* after cryo-preservation in liquid nitrogen (picture taken by Anna Mikuła & Jan J. Rybczyński). The leading article on page 3 focuses on cryo-preservation as a technique to store seeds and spores of native plant species.

02 Phytotronics in seed germination

By Costas A. Thanos*

Germination is an extremely crucial event in the life-cycle of all seed plants as it represents the shift from the most tolerant stage in plant development (dry seed) to the most vulnerable one (early seedling). From antiquity, seed germination has attracted scientific interest especially for crop plants. These were (and still are) subjected to innumerable experiments, with scientific reports from the second half of the 19th century. The initial goal of this work was to achieve as high a percentage germination as possible (in the field and/or laboratory) in the shortest time.

It was quickly observed that obstacles to germination and their underlying mechanisms were inhibiting germination. Subsequently, investigations focused on overcoming these problems became a captivating scientific subject. Achieving successful germination is as important now as it ever was. This pursuit is an important element in *ex situ* conservation. Protocols for specific plant taxa give optimal conditions for germination while ecophysiological studies reveal specific adaptations towards various environmental factors.

Germination *sensu stricto* is a well-defined physiological process. It starts with water absorption and ends with the protrusion of the radicle from the seed. However, it is sometimes more logical to view germination as covering the entire period that transforms seeds into seedlings (the 'germination strategy'), at the right place and in the right time in the field. The germination strategy has been shaped by natural selection and includes numerous adaptations (or adaptive traits) at both structural and physiological levels. This can be studied under controlled conditions by using a phytotron.

A phytotron is a walk-in chamber or a specially constructed room where plant growth is investigated under diurnally programmable conditions. It enables the researcher to precisely administer temperature, light, air

Fig. 2. Diurnal programme of temperature fluctuations and light regimes for a seed germination phytotron. The solid, stepwise curve represents actual temperature values recorded at seed level within Petri dishes while the dotted line represents site recordings in the habitat of *Nepeta sphaciotica*, at 2300 m asl (temperature on soil surface, mean values for September 1-7). The dark area represents the daily skotoperiod while the differently coloured bars correspond to three different levels of light quantity (plus a 30 minute long, far-red enriched light, of lower quantity than the former three levels, applied in the beginning and end of the day)
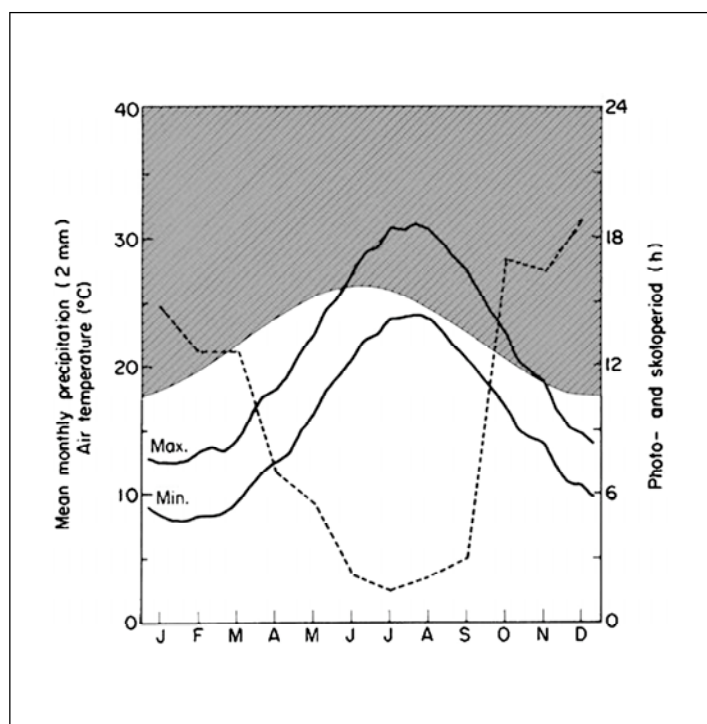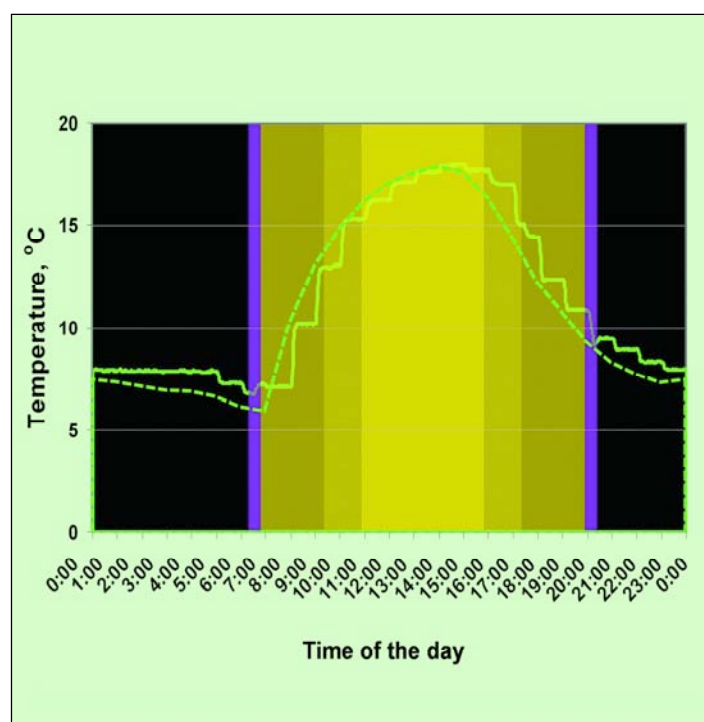

Fig. 1. Climatic data for Athens (Ellinikon Airport). Monthly precipitation values (dotted line) are means for the period 1951-90. Maximum and minimum air temperature values (for each 10-day period) are means between 1955-87. The daily duration of skotoperiod (shaded area) was calculated from official timetables for sunrise and sunset (minus one hour for dawn and dusk light) (Thanos *et al.*, 1991)

humidity, plant substrate, moisture, water potential, CO_2 and nutrient concentrations in order to facilitate their experimental work and reduce environmental variability. Since the study of seed germination virtually involves only the former two parameters (temperature and light), a new generation of plant growth chambers (fully programmable for temperature, light and air relative humidity) are ideally suited to seed germination studies. Additionally, with the advent of inexpensive, yet sophisticated,

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meteorological sensors and data-loggers new perspectives and challenges can be pursued in germination research. For instance, temperature at the soil's surface or at any depth where germination takes place (including stratification temperatures) can now be recorded relatively easily and accurately. Similarly, we are able to monitor soil moisture content that ultimately permits germination. It is also possible to record the relative humidity of the air providing additional data to the amount of rainfall at a given location. Further, rainfall patterns and water availability to the seed (and its duration) may differ, thus having an impact on germination. This effect can be accurately monitored using the data-loggers. Light also varies. The quality and quantity of sunlight along with its photoperiod and the effects of sunflecks and canopy cover can now be monitored experimentally.

In order to carry out ecological investigations on seed germination we need to simulate, as closely as possible, the environmental conditions prevailing in nature at the time of germination. In order to do this the following steps are recommended:

1. Determine the 'germination unit' that will be subjected to experimentation. (not necessarily synonymous to the 'dispersal unit').

2. Define the 'season of germination' (SG) in the field by observing seedling emergence. Identifying the precise 'timeframe' will require monitoring emergence for several consecutive years and allow for annual variation.

3. Discover the microhabitat for seed germination: (a) on or slightly below the soil's surface (e.g. on open ground, under a plant canopy, or in rock crevices – i.e. under 'unfiltered' sunlight, in far-red enriched conditions or in 'neutral-shade' daylight, respectively), (b) at a particular depth beneath the soil (in darkness).

4. Obtain data for the critical climatic parameters during the SG (i.e. temperature and light, Fig. 1), soil moisture and air relative humidity are presumably suitable for germination, provided the SG has been

successfully identified - thus we can ignore 'water availability' from the experimental protocol. However, the latter point is a potential avenue for future investigation, as seeds have been shown to efficiently absorb water vapour (Wuest, 2007).

Temperature values can be acquired from meteorological stations or, even better, obtained from temperature dataloggers that record precise, short-interval readings at the germination microhabitat (ideally recorded during several consecutive years to obtain mean values).

With regard to light, three specific parameters should be taken into account:

a) photoperiod length, easily calculated from official astronomical tables for the particular geographical coordinates (see for example: http://aa.usno.navy.mil remembering to add an additional 30 minutes to cover each pre-sunrise and post-sunset period, dawn and dusk light, respectively.

(b) light quantity, this varies during a single day. However, the amount recorded under clear or homogeneously overcast skies pro-
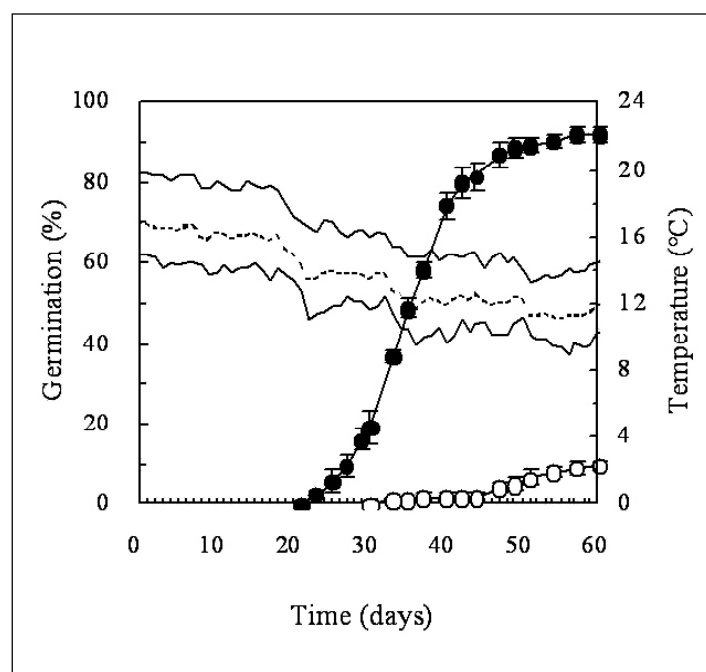

Fig. 4. Germination time course for *Muscari neglectum* under simulated 'November-December' conditions (time 0 corresponds to '1st November'). Lines represent average values for daily temperatures (upper and lower solid lines: max. and min., dotted line: mean). Black circles: continuous darkness; white circles: white light/dark alternations. Vertical bars denote ±SE. (Doussi and Thanos, 2002)

duces a bell-shaped curve for a single day. This data allows light quantity values to be obtained from conventional meteorological data and/or with the use of Photosynthetically Active Radiation, PAR (400-700 nm) and pyranometer (full light) sensors/data-loggers.

(c) light quality, should be measured on site with a spectroradiometer. In addition to the well-known diurnal qualitative variation (particularly during sunrise and sunset where light is significantly
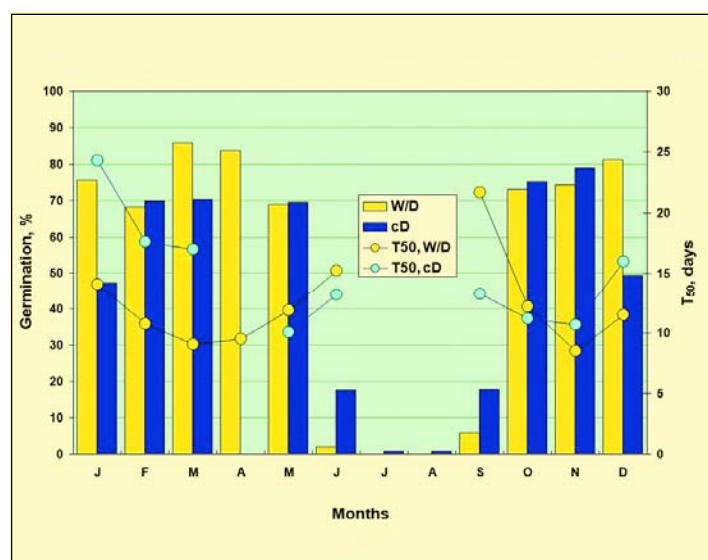

Fig. 3. Final seed germination of *Pinus halepensis* as a function of various temperature and light conditions, simulating those prevailing naturally (Athens, Ellinikon Airport), throughout the year. Shaded bars are controls in continuous darkness. White and grey circles within each pair of bars represent T_{50} values (Thanos and Skordilis, 2003)

enriched in the red (R) and far-red (FR) fractions of the visible spectrum), the light regime below or even near a plant canopy is significantly altered (with the ζ value of light being less the denser the foliage; ζ is defined as the R/FR photon ratio and attains a value between 1.0 and 1.2 under sunlight).

5. Design the simulation programme and carry out the germination experiment(s). On the basis of the data obtained, the phytotron can be programmed with realistic temperature and light conditions (Fig. 2). For the former, hourly steps (or shorter if the instrumentation permits) can sufficiently imitate diurnal variation. For the latter, all three parameters described previously have to be manipulated. In regard to light quantity, we should select a number of different light intensities, representing as closely as possible natural conditions, their duration and sequence. Further, we have to bear in mind that fluorescent light (usually the only light source in plant growth chambers) is quite dissimilar to natural daylight (ζ value around 5.0) and should therefore be supplemented with incandescent light (at least 50% of total wattage has to be provided by the latter; Thanos, 1993). The 30 minute each, dawn and dusk light, should be made solely with incandescent light (strongly imitating natural light at both ends of the day). For special light conditions such as canopy-filtered light a combination of filters (glass, plastic or gelatine) and proper light sources will be needed (see examples in Thanos and Doussi 1995).

6. Include treatments and pre-treatments to seeds prior to or during experimentation. Many plants require additional cues for germination and these may take place in the field either before or during germination. We should always bear in mind that the successful ecological elucidation of a specific germination strategy may need to be integrated with such treatments (e.g. after-ripening, exposure to high temperatures, stratification and the elimination of inhibitors).

Several examples of applying (not always in the same way) the guidelines and concepts previously explained are described below.

• Seeds of *Pinus halepensis* Mill. (Aleppo Pine) were imbibed under temperature and light conditions simulating, on a monthly basis, those occurring naturally, throughout the year, in a typical Mediterranean site. Seeds kept in continuous darkness showed a slower rate of germination and in several cases germination decreased. Near-optimal germination was obtained during the relatively 'cool' months where water is more or less available in the field. (Fig. 3).

• Seeds of the geophyte *Muscari neglectum* were imbibed under conditions simulating, on a daily basis, those met during November and December. Despite a very slow rate, those germinated under dark conditions gave optimal and nicely sigmoid germination; on the other hand, a strong photoinhibition was found, implying that these seeds have adapted to germinate only when buried in the soil (Fig. 4).

• Seeds of the critically endangered, Cretan endemic *Nepeta sphaciotica* were subjected to conditions shown in Fig. 2, in the light (the species is an absolute light requirer). Under the present conditions germination is minimal but if seeds are transferred to a warmer regime (as predicted by the climate change scenario B2a), germination is significantly enhanced. Since this taxon is adapted to germinate when the snow has thawed (in May-June) precocious germination prior to the almost six-

month long snow cover period would certainly minimise any chances of seedling survival and, thus, trigger the species extinction. These experiments were carried out in the context of the Interreg Project SEMCLIMED. (Fig. 5).
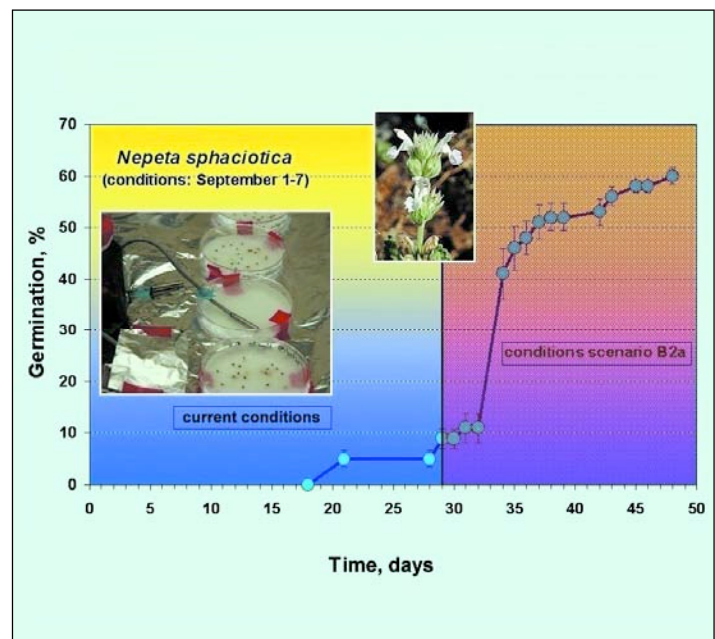

Fig. 5. Seed germination rate of *Nepeta sphaciotica* under simulated, present day (left) and for future predictions (right). Insert (left) shows a Petri dish and sensor monitoring temperature at seed level. Insert (right) are the flowering heads of this species (Thanos *et al.*, 2008 unpublished)

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